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Sediment Bacterial Indicators in an Urban Shellfishing Subestuary of the Lower Chesapeake Bay

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The objectives of this study were to document the spatial and temporal distributions and compositions of bacteria in the sediments and overlying waters of an important urban shellfishing area in the lower Chesapeake Bay region, the Lynnhaven Estuary. Marked fluctuations were observed in the data of many of the physicochemical parameters and the indicator bacteria. The higher-salinity water and coarser sediment of the inlet site showed lower overall bacterial densities than did the headwater sites, where freshwater runoff and decreased tidal action were characteristic. Densities of benthic indicator bacteria, when expressed on a volumetric basis, were significantly greater than counts in the overlying waters. These counts were indicative of a fecally polluted system and were well above the safe maximum limits for shellfish-growing waters. Significantly fewer total and fecal bacteria were observed in both the water and the sediment during the warm months of May, July, and August. The primary sources of the Lynnhaven's bacterial pollution appeared to be typical of urban and agricultural runoff, although failure of septic tank systems was suspected as a problem in the Lynnhaven's western branch. These results illustrated that sediments in shellfishing areas could serve as a reservoir for high densities of indicator bacteria and that, potentially, pathogens could pose a health hazard.

A considerable amount of literature is available concerning the development of methodologies and the distribution of indicator bacteria in aquatic environments. Studies dealing with the estuarine environment have been limited, and only a few have been published on research conducted in estuarine sediments (15, 24). Furthermore, microbiological studies done in the lower Chesapeake Bay and its urban subestuaries are almost nonexistent. As a result, a yearlong investigation was completed in the Lynnhaven Estuary, and this initial study represents the first phase in accumulating a comprehensive bacterial data base for this important urban shellfishing area of the lower Chesapeake Bay. Information derived from field and laboratory studies is being applied in management decisions concerning land use and development in the Lynnhaven's drainage basin. The scientific data are being used in part to enhance our understanding of bacterial pollution indicators, the microbial ecology of fecal and native bacteria, the value of fecal coliforms as a tool in detecting the presence of fecal and nonfecal pathogenic bacteria in shellfish-growing waters, and the impact of land development on the water quality of the urban estuarine environment.

MATERIALS AND METHODS

Environmental setting. The Lynnhaven Estuary (37°53′ N, 76°05′ W) is an urban subestuary of the lower Chesapeake Bay. It is a temperate, well-mixed estuary in which tidal flow dominates near the inlet to Chesapeake Bay but is considerably reduced in the headwaters. The major sources of freshwater inflow to the system are primarily rainwater runoff, groundwater intrusion, and low river flow. The estuary has a typical tidal amplitude of about 61 cm, and the two branches of the Lynnhaven River—the eastern and western branches—have a wide variety of marsh vegetation, including *Spartina*, *Juncus*, *Phragmites*, *Baccharis*, and *Iva*. The average depth of the water at mean low tide is about 76 cm.

Sampling sites. Eleven sampling sites were located throughout the estuary from the inlet to the headwaters of both the eastern and the western branches of the Lynnhaven (Fig. 1). These sites were situated to provide good spatial distribution throughout the estuary. All sediment sampling sites were located subtidally, in waters with depths ranging from 0.51 to 2.28 m throughout the entire system during the sampling period.

Samples were collected in triplicate at least bimonthly. Grab samples of subsurface water were collected in sterile glass widemouthed bottles before sediment sampling and immediately placed on ice. Samples were collected with care so as not to disturb the sediment while sampling in the shallow headwaters. A

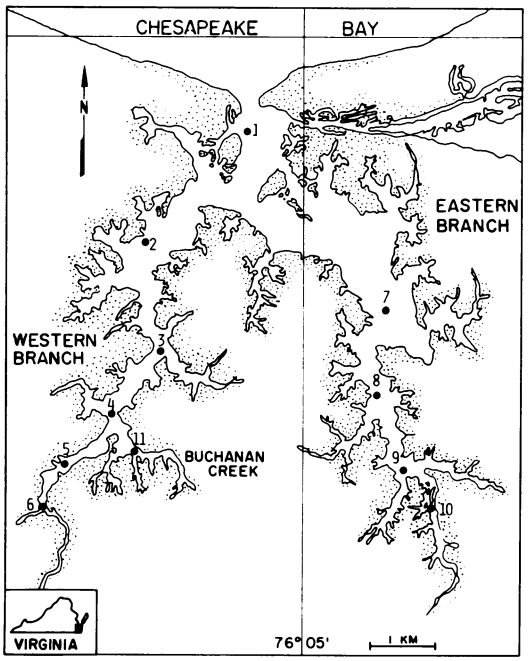

FIG. 1. *Map of the Lynnhaven Estuary, showing the distribution of the 11 sampling sites along the eastern and western branches of the Lynnhaven River.*

hand-held coring device was used to retrieve primary sediment cores. Each primary core was immediately subsampled twice; one subcore was transferred to a sterile, precalibrated bottle for bacteriological analysis, and the other was placed in a clean, preweighed aluminum pan for dry weight analysis. Both were returned on ice to the laboratory. Total elapsed time from the beginning of sampling to initiation of analyses in the laboratory did not exceed 6 h. All samples were collected during the day at or near high tide because of the limited boat accessibility of the sites on the headwaters of the Lynnhaven River branches.

Bacterial analyses. Procedures for the enumeration of total and fecal coliforms and fecal streptococci from water followed the five-tube fermentation technique as specified in the 14th edition of *Standard Methods for the Examination of Water and Wastewater* (1). Lauryl tryptose broth was used as the medium for presumptive enumeration of total coliforms. Fecal streptococci were verified microscopically by examining stained smear preparations as suggested by Buck (3).

Total viable counts of the aerobic, heterotrophic bacteria were determined by a serial dilution-spread plate technique, using a seawater-yeast extract medium (31). Visible colonies were counted after 7 days of incubation at room temperature.

Total and fecal coliforms, fecal streptococci, and aerobic heterotrophs were enumerated from sediments by the same basic methodologies used for the water samples. Sediment subcores were diluted to 100 ml with sterile phosphate buffer and blended at high speed for 2 min. Aliquots of the diluted sample then were either dispensed directly into multiple fermentation tubes for coliform and streptococcal analysis or serially diluted and plated on seawater-yeast extract media for total viable counts.

Physicochemical analyses. Total, volatile, and fixed suspended solids were gravimetrically determined by filtering 100 to 400 ml of sample through preignited and tared glass fiber filters by the methods outlined by Millipore Corp. (27).

Sediment dry weight was obtained by drying each sediment subcore at 100°C for 3 days, cooling it, and reweighing it.

In situ salinity and water temperature were monitored with a portable, battery-powered Beckman RS5-3 salinometer, whereas sediment temperature was measured with a hand-held mercury thermometer. Dissolved oxygen was measured in situ with a Yellow Springs Instruments model 54 dissolved oxygen meter calibrated in the field by the water-saturated air technique and later corrected for in situ salinity. The pH values of all water samples were measured in the lab with a Corning model 7 pH meter, and values were corrected for in situ water temperature (36).

Water depth was directly measured by carefully lowering a weighted calibrated nylon rope to the sediment surface. Precipitation data were obtained from the meteorological section of the Oceana Naval Air Station, Virginia Beach, Va.

RESULTS AND DISCUSSION

Historically, the Lynnhaven, an urban shellfishing estuary of the lower Chesapeake Bay region, has been opened and closed periodically to shellfishing during the past 40 years due to high fecal coliform counts. Today, all point sources of pollution have been eliminated except the Birchwood Garden sewage treatment plant, whose effluent empties into Buchanan Creek in the western branch of the Lynnhaven River (Fig. 1, site 11).

Although several agencies routinely monitor the waters of the Lynnhaven for ambient water quality and shellfish stock, no comprehensive investigation of the bacteriology of this estuary has ever been undertaken. Consequently, our yearlong study of the Lynnhaven was initiated not only to bridge the gap in our knowledge of the system but also to investigate the benthic environment of this important shellfishing area as a potential reservoir for microorganisms commonly considered indicators of water quality.

The Lynnhaven is classified as a small coastal river basin of the Chesapeake Bay system. The outstanding physical feature of the Lynnhaven River complex is its division into two major branches, the eastern and western branches.

Based primarily on salinity patterns, this estuary would probably be categorized as a well-mixed estuary in which tidal flow dominates. Poor tidal flushing in the headwaters and the mixing of brackish and fresh waters have resulted in a longitudinal gradient characterized by salinities approaching that of the bay near the inlet to near freshwater in the headwaters (21). Although the pattern was consistent throughout the year, the magnitudes of the monthly averages were dependent upon the amounts of rainfall and subsequent runoff. The horizontal, tidal, and seasonal fluctuations of salinity and the other measured physicochemical parameters (Table 1) illustrate well the heterogeneity that is characteristic of this and other tidal estuaries along the East Coast.

With respect to Virginia's water quality standards for class IIB waters, the average yearly values of water temperature (15.9°C), dissolved oxygen (7.64 mg/liter), pH (7.62), and suspended solids (49.75 mg/liter) were well above the minimum acceptable limits established by the Virginia State Water Control Board (40). The total suspended solids load, long considered a major nonpoint pollutant, was only 62.5% of the reference level for aquatic life (80 mg/liter) suggested by the Environmental Protection Agency (6). However, occasional high averages were noted in March (86.8 mg/liter) and May (74.2 mg/liter) and illustrate well the siltation problem that plagues this system. It is ironic that the levels of total suspended solids in the Lynnhaven's populated drainage basin were nearly three times less than total suspended solids values monitored in a so-called pristine, remote North Inlet estuary near Georgetown, S.C. (C. W. Erkenbrecher, Ph.D. thesis, University of South Carolina, Columbia, 1976). The compositions of the suspended materials in the waters of the two estuaries also differed considerably; the level of volatile suspended solids in the Lynnhaven (30.6%) was almost twice that of the North Inlet (18.5%).

A summary of all bacteriological data collected during this study is presented in Table 2. Because of the inherent problems of comparing waterborne coliform counts (most probable number per 100 ml) to benthic counts (most probable number per gram, dry weight), all sediment coliform and streptococcal data were also calculated and recorded on a volumetric basis (i.e., most probable number per 100 cm^3 of sediment). Only on this basis could the aquatic and benthic densities be directly compared. This approach was also used by Goyal et al. (15) and Hussong et al. (22) in analyzing aquatic and benthic samples from Texas canals and the upper Chesapeake Bay area, respectively. Throughout the study, bacterial densities in the water and sediments demonstrated considerable variability, as seen by the extreme ranges of values reported. The densities of sediment bacteria were, on the average, a couple to several orders of magnitude higher than corresponding counts in the water when directly compared on a volumetric basis (i.e., most probable number per 100 ml compared with most probable number per 100 cm^3). It should be emphasized that the bacterial densities recorded throughout this study were probably conservative (i.e., minimum range) estimates of the true mean densities, because all samples were collected at or near high tide. The influence of tidal flux on bacterial densities has previously been demonstrated by Buck (3) and Erkenbrecher and Stevenson (7).

The trends in spatial distribution of waterborne total and fecal coliforms, fecal streptococci, and the native aquatic bacteria throughout the Lynnhaven Estuary were strikingly sim-

TABLE 1. *Results of physicochemical analyses in the Lynnhaven Estuary*

Statistic	Variable														
	Depth (m) (N^a = 225)	Temp (°C) (N = 225)			Salinity (‰) (N = 225)	Oxygen (N = 216)		pH (N = 225)	Rainfall (cm) over preceding:		Suspended solids (mg/liter)			Sediment wt (g)	
		Air	Water	Sediment		Dissolved (ppm)	Saturation (%)		24 h (N = 225)	5 days (N = 225)	Total (N = 219)	Volatile (N = 188)	Fixed (N = 187)	Dry (N = 223)	Wet (N = 162)
Mean	1.19	15.90	18.43	18.56	14.32	7.64	100.23	7.62	0.26	1.64	49.75	15.23	39.78	1.84	4.13
Standard deviation	0.37	6.78	7.55	7.75	7.17	2.78	27.91	0.56	0.32	1.89	29.62	11.94	27.60	1.08	0.81
Range															
Low	0.51	0.30	1.90	2.00	0.09	2.83	38.80	4.20	0.00	0.00	1.50	0.50	1.00	0.79	2.73
High	2.28	23.90	29.20	28.30	28.90	13.48	157.30	8.90	1.00	5.30	148.00	79.50	129.90	5.44	7.14

[a] N, Number of observations.

TABLE 2. *Summary of all bacteriological data collected from the 11 sampling sites located throughout the Lynnhaven Estuary*

Statistic	Total viable counts		Total coliforms (N^a = 225)			Fecal coliforms (N = 225)			Fecal streptococci (N = 225)			Fecal coliforms/fecal streptococci	
	Water (no./ml × 10^2) (N = 214)	Sediment (no./g [dry wt] × 10^2) (N = 222)	Water (MPN[b]/100 ml)	Sediment		Water (MPN/100 ml)	Sediment		Water (MPN/100 ml)	Sediment		Water	Sediment
				MPN/100 cm^3	MPN/g (dry wt)		MPN/100 cm^3	MPN/g (dry wt)		MPN/100 cm^3	MPN/g (dry wt)		
Geometric mean	—[c]	—	2,897	132,130	2,884	489	38,548	807	805	103,276	2,148	0.6	0.4
Mean[d]	566	1,525	26,233	1,047,433	30,127	4,004	289,542	8,925	7,621	603,748	19,066	0.5	0.5
Range													
Low	1	3	14	67	1	2	67	1	12	67	1		
High	24,000	12,212	240,000	53,328,000	813,001	240,000	311,890,000	196,270	240,000	30,636,000	974,886		

[a] N, Number of observations.
[b] MPN, Most probable number.
[c] —, Data not available.
[d] Arithmetic mean calculated according to Thomas (37), using log-transformed MPN values.

ilar (Fig. 2 through 4). Densities of indicator and native bacteria were consistently lower adjacent to Lynnhaven Inlet than at stations in the upper, landward reaches of the eastern and western branches. Bacterial distributions compared favorably with the observed longitudinal salinity gradient; the salinity of the water was repeatedly higher at the sites with the lowest coliform counts. This was also observed by Pike et al. (30). The relationships between coliforms and salinity, water temperature, and pH suggested that the combined effects of dilution (2, 23) and the bactericidal properties of seawater were responsible for the reduction in counts near the inlet. A similar relationship also has been reported by Ogawa (28) for the estuary of the Tomoe River, Japan. Faust et al. (9) provided supportive data by observing an inverse correlation between the survival of *Escherichia coli* and the salinity of water. In addition, a whole host of other physical, chemical, and biological

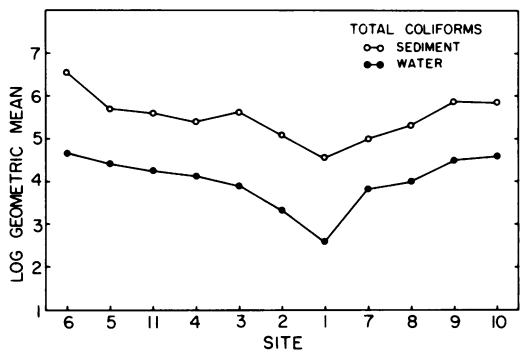

FIG. 2. *Spatial distributions of total coliforms in sediment and water samples collected throughout the Lynnhaven Estuary. Each point was derived from a compilation of all data for that site for the entire yearlong study.*

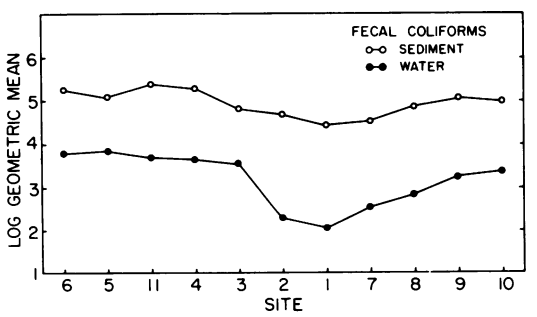

FIG. 3. *Spatial distributions of fecal coliforms in sediment and water samples collected throughout the Lynnhaven Estuary. Each point was derived from a compilation of all data for that site for the entire yearlong study.*

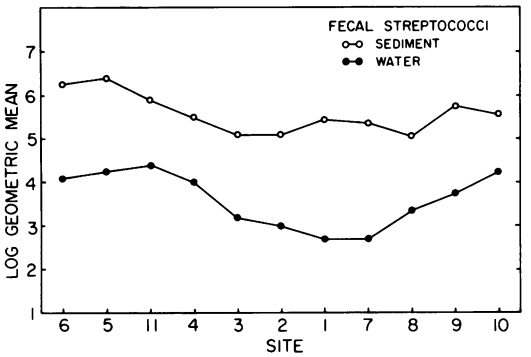

FIG. 4. *Spatial distributions of fecal streptococci in sediment and water samples collected throughout the Lynnhaven Estuary. Each point was derived from a compilation of all data for that site for the entire yearlong study.*

factors have been reported to contribute to the mortality of coliforms in brackish or marine waters (29, 39).

In the less saline headwater areas, there was a corresponding increase in the densities of coliforms and fecal streptococci. The increased concentrations of total suspended solids were associated with peak densities of fecal streptococci and total coliforms, but not with waterborne fecal coliforms. Because of their known associations with plants and soils (10, 12, 13), it seemed reasonable that total coliforms and fecal streptococci were a reflection of the urban runoff that is so characteristic of this system. It should be pointed out that at no time were the total coliform counts in Buchanan Creek, at site 11, inconsistent with the expected counts for that region of the estuary.

The observed high levels of fecal coliform contamination, however, were probably the result of a combination of several factors in which domestic septic tanks or drainage field failures have been implicated. The soils of the headwater areas are classified as well to moderately well drained by the U.S. Department of Agriculture's Soil Conservation Service, with water tables ranging from 0.9 to greater than 1.2 m below the ground surface. During periods of heavy rainfall, septic tank leachate could provide a substantial inoculum of fecal coliforms into the adjacent tidal creeks (17). The most important factor, however, has been overlooked for decades because of the restrictive water sampling regime established by water quality agencies. Roper and Marshall (33) demonstrated that fecal coliforms (especially *E. coli*) can desorb from benthic sediments under conditions of reduced salinity. Because dramatic fluctuations in salinity occur in the headwaters of the Lynnhaven, desorption has become a highly plausible explanation for why fecal counts increase often after significant freshwater flow into these areas of the Lynnhaven. This conclusion was supported by a very strong correlation between waterborne and sediment fecal coliform levels ($r = 0.86$, significant at the $P = 0.01$ level). Likewise, lower fecal counts in the eastern branch were no doubt associated with overall higher salinities and better soil drainage characteristics than those of corresponding areas in the western branch.

One of the most striking observations made during this yearlong study was that the densities of all indicator bacteria were always significantly higher in the sediments than in the overlying subsurface waters (Fig. 2 through 4). The magnitude of the difference was as much as several logs higher for coliforms and fecal streptococci in the sediment, irrespective of the time of year. Although several investigators have also noted inflated benthic coliform densities in river (16, 25, 38), coastal (22, 32), and canal (14, 15) community sediments, this is the only known intensive field study conducted in coastal shellfish-growing waters and sediments. Unlike the more stable bacterial densities noted in Texas canal sediments by Goyal et al. (15), the densities of bacteria in the upper few centimeters of sediment throughout the Lynnhaven fluctuated with the observed spatial and temporal variability in the overlying waters. From horizontal studies, it appeared that many of the same factors controlling the distribution of indicators were also influencing sediment densities. However, in addition to the controlling effects of salinity, dilution, and the bactericidal properties of seawater, to mention a few, the low organic content of the coarser sediments (4) and the stronger tidal currents in the inlet may be the major reasons why bacterial counts were lowest there. In fact, Gerba and McLeod (14) and Savage (34) demonstrated that the extended survival of *E. coli* in sediments depended on a greater content of organic matter in the seawater.

In the headwaters, higher benthic bacterial counts were suspected to be the result of replenishment or multiplication. The major contributing factors of replenishment are sedimentation of suspended solids and attached microorganisms originating from urban runoff during periods of heavy rainfall. Matson et al. (25) suggested that decreased counts in the water were associated with increased counts in the sediments and were not necessarily just from die-off. Other investigators have also favored the importance of sedimentation in describing reductions in coliform levels in water (23, 28, 41). In addition to the protective mechanisms described by Roper and Marshall (33), the sediments have been

shown to provide a suitable environment for the survival and growth of indicator bacteria.

In this connection, Ogawa (28) and Hendricks (18) have pointed out that longer survival is directly related to an increase in organics in sediments over those in water; however, variability in survival of *E. coli* also depends on the types of nutrients available (14). Sediment eluate studies have shown growth of *E. coli* (14), *Enterobacter aerogenes*, and the pathogenic bacteria *Shigella*, *Salmonella*, and *Arizona* (18–20), and this growth has taken place in some instances despite the presence of other competing microorganisms. Thus, it has been demonstrated that not only do the sediments harbor significantly high numbers of indicators (i.e., act as reservoirs), but also the sediment environment has the potential to protect and provide nutrients for the growth of indicators and pathogenic bacteria. In two associated studies, it was demonstrated that in most cases the fecal coliform levels in oysters collected throughout the Lynnhaven were consistently higher than waterborne counts (unpublished data). If fecal bacteria are indeed released to the overlying waters (and it appears that this may be the case), then these contaminated sediments could pose a serious threat to the health of individuals ingesting shellfish from these areas or coming into direct contact with the waters through recreational and other activities.

Numerous investigators have reported seasonal temperature influences from field studies and temperature-dependent survivability studies in the laboratory on indicator bacteria. Overall, greater bacterial densities have been observed during the winter months than in the summer (9, 15, 35). Survival of *Enterobacteriaceae* was shown by McFeters and Stuart (26) to be inversely related to seasonal temperature, especially below 15°C. Faust et al. (9) further demonstrated a rapid decrease in *E. coli* survival at elevated temperatures in estuarine environments. These studies, however, are not in general agreement with the observed seasonal occurrences of indicator bacteria in the Lynnhaven Estuary; that is, peak densities of total and fecal coliforms and fecal streptococci occurred in early spring (March) and late summer (August) (Fig. 5). Although significant associations between coliforms and water temperature were apparent, the dominant factor in the Lynnhaven influencing the peak aquatic densities was heavy rainfall associated with a concomitant increase in storm water runoff during the 24-h period before sampling in both instances. Faust (8) noted that, in a similar fashion, the rates of coliform discharge were dependent on the water flow characteristics of the estuary of the Rhode River, Md. The

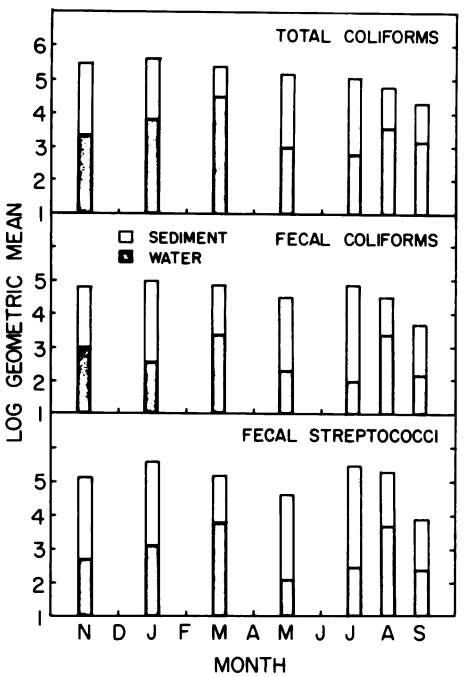

FIG. 5. *Seasonal fluctuations of total and fecal coliforms and fecal streptococci in the sediment and water of the Lynnhaven Estuary. Each bar represents a composite of all data for the entire 11 sites during the yearlong study.*

significance of storm water runoff has also been demonstrated by Geldreich et al. (11). The seasonal variability of the sediment bacteria in the Lynnhaven was similar to those described in some of the previously mentioned studies in that maximum densities were recorded for coliforms and fecal streptococci in the winter (January) (Fig. 5). Supportive studies, on Shimizu Harbor and Orido Bay, Japan, have been reported by Ogawa (28).

Associated with the observed high benthic coliform counts throughout the estuary is the potential for the disturbance and resuspension of these contaminated sediments into the water column. This message of caution is no doubt applicable to most urban estuaries having high fecal coliform densities and has been suggested by studies in Connecticut (25) and Texas (15). The physical mechanisms that could result in the resuspension of the upper few centimeters of mud are almost universal in most coastal ecosystems: (i) strong tidal current velocities; (ii) wind-induced turbulence and storm activity; (iii) recreation activities, such as boating, swimming, and water-skiing; (iv) normal macroorganism activities by bottom-feeding fish and invertebrates; and (v) maintenance dredging operations. In the

Lynnhaven Estuary, additional mechanisms related to commercial shellfishing and marina activities are also important. Although no data have been published as yet relating resuspension of contaminated bottom sediments with release of bound coliforms and pathogens into the water, the point may be moot in that bacteria released by any of the above-mentioned mechanisms would surely be detected in any water samples collected at the time of resuspension. On the other hand, as Matson et al. (25) point out, "observed or predicted reductions in waterborne indicators should not be the sole criterion for determining potential health hazard presence, since many viable organisms (coliforms and pathogens) are deposited in the sediments."

We are aware of the problems inherent in interpreting fecal coliform/fecal streptococcus ratios, one of which is the high ratios associated with wild migratory birds (22). However, significant numbers of migratory birds were not observed during this study and are usually not present in this ecosystem. Despite the shortcomings of such an evaluation, the fecal coliform/fecal streptococcus ratios do illustrate a significant difference between the eastern and western branches (Fig. 6). Overall, sporadic high fecal coliform/fecal streptococcus ratios throughout the western branch strongly implicated human influences on the system. Especially notable were the high ratios observed at sites 4 (6.4) and 6 (4.1) and the lowest overall ratio for the western branch at site 11 (1.3), which monitored the Birchwood Gardens sewage treatment plant. The eastern branch and the inlet, in contrast, exhibited low ratios, around 1.0, indicative of a predominance of animal wastes in mixed pollution. The somewhat surprising differences between the two branches of the Lynnhaven indicate that failing septic tank systems in only moderately well drained soils and, probably, discharges of raw or poorly treated sewage from the numerous boats in the area appear to be the origins of the pollution in the western branch. Unlike the western branch, the eastern branch is characterized by much more vacant and agricultural land and fewer single-family residences. This translates into reduced fecal loads because of fewer septic tank systems (and associated failures), a greater potential for increased total coliforms and enterococci due to storm water runoff, especially from the vacant and agricultural land, and significantly lower overall fecal coliform/fecal streptococcus ratios.

In short, from the data collected during this yearlong investigation of the Lynnhaven Estuary, it is clear that the major problems inherent in this system are nonpoint in their origin. The

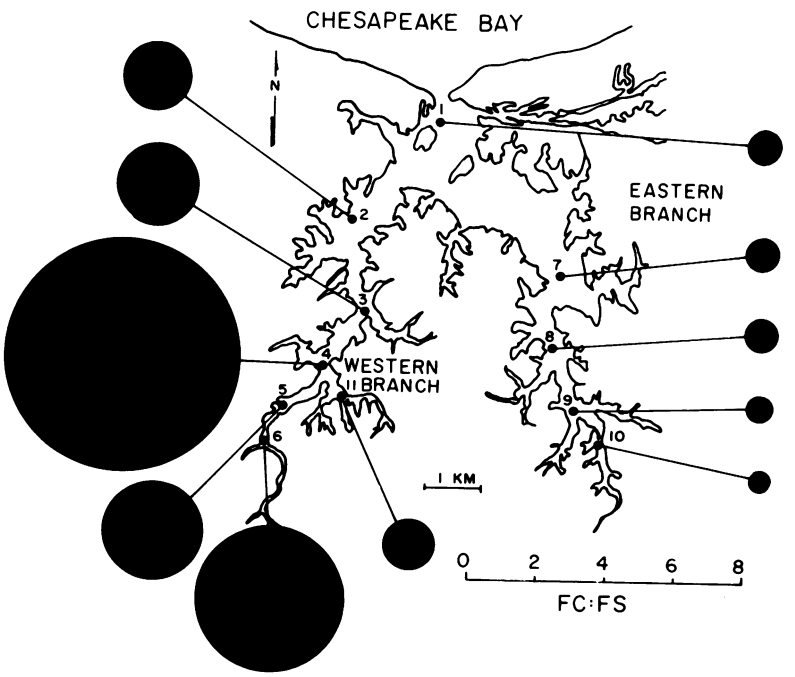

FIG. 6. *Map illustrating the spatial distribution of the overall fecal coliform/fecal streptococcus ratios for each site throughout the Lynnhaven. The diameter of each darkened circle is equivalent to the ratio.*

apparent difficulties previously encountered in trying to identify the major nonpoint sources through analyses of the transient water may be minuscule compared with the potentially hazardous high levels of fecal bacteria in all sediments throughout the Lynnhaven. These elevated sediment densities of fecal coliforms were also found associated with oysters from both branches. The single most important source of fecal pollution entering the system appears to be the failure of septic tank systems and drainage fields to cope with the high water table and heavy rainfall that is typical of the area. Additional sources of nonpoint fecal pollutants that deserve closer examination include waste disposal and discharge from the numerous private and commercial boats in the area; the impact of migratory waterfowl, gulls, and wild and domesticated animals; the seepage of poor-quality or even contaminated groundwater from artesian aquifers (5; L. Tluczek and E. Carlson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, N19, p. 187); and the semidiurnal intrusion of potentially poor-quality water from the lower Chesapeake Bay.

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